F ENT COOPERATION TREA.

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

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ROYAUME-UNI

Date of mailing (day/month/year)
18 September 2000 (18.09.00)

Applicant's or agent's file reference
PHM/70429/WO

IMPORTANT NOTIFICATION

International application No.
PCT/GB99/03789

International filing date (day/month/year)
12 November 1999 (12.11.99)

1. The following indications appeared on record concerning:

[ ] the applicant [ ] the inventor [X] the agent [ ] the common representative

Name and Address

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2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address

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Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

I. Britel

Telephone No.: (41-22) 338.83.38

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
PHM/70429/WO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 99/03789	12/11/1999	17/11/1998
Applicant		
ZENECA LIMITED et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 2 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[X] contained in the international application in written form.

[X] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[ ] the text is approved as submitted by the applicant.

[X] the text has been established by this Authority to read as follows:

METHOD FOR IDENTIFYING INHIBITORS OF IPC SYNTHASE

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1a

[ ] as suggested by the applicant.

[X] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[ ] None of the figures.

PATENT COOPERATION TREATY

REC'D 04 DEC 2000

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

15

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
PHM.70429/WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/03789	12/11/1999	17/11/1998
International Patent Classification (IPC) or national classification and IPC		
C12N15/81		
Applicant		
ASTRAZENECA UK LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
16/05/2000	29.11.2000
Name and mailing address of the international preliminary examining authority:	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Paresce, D Telephone No. +49 89 2399 8995

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/03789

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-6 as originally filed

Claims, No.:

1-7 as originally filed

Drawings, sheets:

1/2-2/2 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 4-6
	No:	Claims 1-3, 7
Inventive step (IS)	Yes:	Claims
	No:	Claims 4-6
Industrial applicability (IA)	Yes:	Claims 1-7
	No:	Claims

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

R It m V

Reason d statement under Article 35(2) with r gard to n v lty, inv ntiv st p or industrial applicability; citations and explanations supp rting such stat m nt

1) Reference is made to the following documents:

D1: M M NAGIEC ET AL: 'Sphingolipid synthesis as a target for antifungal drugs' JOURNAL OF BIOLOGICAL CHEMISTRY,US,AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 15, no. 272, 1 January 1997 (1997-01-01), pages 9809-9817, XP002077632 ISSN: 0021-9258 cited in the application

D2: EP-A-0424117

D3: US-A-5667986

2) Novelty: Article 33(2) PCT

The subject-matter of claims 1-3, 7 is not considered new in the sense of Article 33(2) PCT for the following reasons:

D1 describes an experimental approach for isolating strains defective in the later steps in sphingolipid biosynthesis that involves the use of a sphingolipid compensatory (SLC) strain. The SLC strain used (7R6) carries two mutations, a deletion of the lcb1 gene and a point mutation that creates the suppressor gene SLC-1. This mutant isolation procedure allowed the isolation of a mutant *S. cerevisiae* strain defective in IPC synthase activity. D1 discloses the complementation of an IPC synthase gene defect in the mutant strain of *S. cerevisiae* by the AUR1 gene. Mutations in AUR1 had been shown previously to give resistance to the antifungal drug aurobasidin A (AbA). D1 discloses that AbA was shown to be a potent inhibitor of IPC synthase activity. It is postulated that it should be possible to develop high throughput screens to identify new inhibitors of IPC synthase to combat fungal diseases (see abstract and introduction).

The IPEA is of the opinion that the screening assay, SLC strains and the IPC synthase inhibitor disclosed in D1 would fall under the scope of claims 1-3, 7 of the present application.

The subject-matter of claims 4-6 has not been made available to the public by any of the available prior art documents and can therefore be regarded as novel.

3) **Inventive Step: Article 33(3) PCT**

The subject-matter of claims 4-6 is not considered to involve an inventive step in the sense of Article 33(3) PCT for the following reasons:

D1 is regarded as being the closest prior art to the subject-matter of these claims. The subject-matter of claims 4-6 consists in the provision of cells that are slightly different from those of D1. The dominant SLC-1 allele was generated from the wildtype by PCR regenerating the sequence described in D1. From D1 it was expected that the SLC-1 mutation should allow growth of the mutant strains on media without added phytosphingosine. It is stated in the present application that the viability of the resulting lcb1::kanMX was, however, extremely poor. It was postulated that the poor viability of the lcb1::kanMX strain could be due to insufficient expression of SLC-1 so increased expression of SLC-1 was attempted. The SLC-1 gene was placed under the control of the GPD3 promoter. The use of a multi-copy promoter GPD-SLC-1 promoter/gene construct yielded a strain with much improved growth characteristics and resistance to freezing.

The IPEA is of the opinion that the use of a known promoter (see D2 or D3), to increase expression of a known gene would come within the scope of the customary practice followed by persons skilled in the art. The selection of the GPD3 promoter rather than other known yeast promoters such as PGK, ENO, PYK, as listed on p.3 of the present application, can only be regarded as inventive, if said cells presented unexpected effects or properties in relation to the other cells disclosed in D1. At present, an inventive step for the cells cannot be recognized, unless said yeast strain will show some kind of unexpected advantages over those described in prior art, which should be demonstrated.

VIII. Certain observations on the international application

1) **Clarity: Articl 6 PCT**

Claim 4 is not clear because it refers to a SLC-1 gene under the control of the GDP3 gene. It is unclear whether this includes the full-length GPD3 gene or just the GPD3 promoter. Also the claim should probably read, GPD3 and not GDP3.

Claim 6 is not clear. The use of internal arbitrary designations of cell lines are meaningless to the person skilled in the art and do not constitute a definition through technical means as required by Article 6 PCT. These cells should be, generally, clearly and unambiguously characterized e.g. by reference to technical features, or by reference to accession numbers of a deposit, in order to satisfy the requirements of Article 6 PCT.

FOR THE PURPOSES OF INFORMATION ONLY

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METHOD FOR IDENTIFYING INHIBITORS OF IPC SYNTHASE

The present invention relates to a cell-based screen for inhibitors of fungal inositolphosphoryl-ceramide (IPC) synthase, an important antifungal target.

Inhibitors of fungal IPC synthase are potent and selective antifungal agents for example Aureobasidin, Khafrefungin and Rustmicin) as identified by several research groups and pharmaceutical companies.

However, all such compounds are natural products that are difficult to produce, handle and administer to a patient (for example, they may have unsuitable pharmacokinetics). Therefore it is highly desirable to obtain other novel chemical compounds selectively inhibiting the same target (a fungal IPC synthase) but without the intrinsic disadvantages displayed by the currently known inhibitors. Screening for such novel chemicals as well as optimisation of already available "leads" (ie. optimisation of a known inhibitor in a structure-based design or lead optimisation) will require an assay for IPC synthase activity that can be performed at a sufficiently high throughput.

All currently available biochemical assays for IPC synthase are involved and very labour-intensive.

Nagiec et al (Journal of Biological Chemistry, Vol 272 No 15, pp 9809-9817 (1997))) describe the complementation of an IPC synthase gene defect in a mutant strain of *S. Cerevisiae* by the *AUR1* gene. The mutant strain has a deletion of the LCB1 gene and a point mutation that creates the suppressor gene SLC1-1. The lcb1 mutation prevents sphingolipid synthesis and the SLC-1-1 gene enables the cells to make phospholipids and remain viable. (Use of capital letters implies a functional gene or a gain of function mutation such as SLC1-1 whereas small letters indicate a non functional allele such as lcb1). Using this the authors were able to isolate a mutant strain defective in IPC synthase and to isolate a gene AUR1 which complemented the IPC synthase defect and restored IPC synthase activity. The authors conclude that IPC synthase is the target for antifungal agents such as aureobasidin. They postulate that it should be possible to develop high throughput screens to identify new inhibitors of IPC synthase to combat fungal diseases.

However we have found that whilst a similar strain of *S. cerevisiae* (lcb1 / SLC1-1) is viable, the strain grows very poorly and is extremely sensitive to any environmental

influences such as for example freezing. This strain is simply not robust enough for screening purposes.

We now provide a robust cell-based assay for identifying selective IPC synthase inhibitors. This assay is based on our development of an *S. cerevisiae* strain wherein the production of compensatory phospholipids is enhanced.

Therefore in a first aspect of the present invention we provide a screening assay for identifying a selective IPC synthase inhibitor which assay comprises contacting a test compound with engineered cells whose capability to synthesize sphingolipids depends on the addition of exogenous phytosphingosine and which are capable of sustained growth via compensatory phospholipids, adding phytosphingosine, and determining IPC synthase inhibition by the test compound by reference to any cell growth inhibition.

Any convenient host cell strain may be used provided that it can function as a host for a fungal IPC synthase gene. Convenient hosts include fungi that are manipulatable genetically such as *S. cerevisiae* but also others such as Candida albicans, Candida glabrata, Aspergillus sp. or Schizosaccharomyces pombe. Convenient sources for the AUR1 gene are pathogenic (also phytopathogenic) fungi as outlined above and others such as Ashbya sp., Fusarium sp., Trichoderma sp., Cryptococci, Blastomyces, and Histoplasma.

Whilst we do not wish to be bound by theoretical considerations the compensatory phospholipids are believed to be novel glycerophospholipids that may compensate for one or more functions of sphingolipids essential for vegetative growth (Lester et al, J.Biol.Chem., 1993, 268, 845-856).

In a further aspect of the invention we provide engineered cells whose capability to synthesize sphingolipids depends on the addition of exogenous phytosphingosine and which are capable of sustained growth via compensatory phospholipids

By "sustained growth" we mean no significant decrease of viable cell counts during a growth period (ie. cell-death is negligible compared to cell growth). The strain also has to be capable of one or more of the following: being stored for prolonged periods, for example up to three or six months or longer; storage in liquid medium; or capable of being frozen and revived. The engineered cells of the invention are capable and robust enough for routine use in high throughput assay procedures. In general they will have generation times compatible with growth assays (ie. not more than 4 hours per doubling) and final optical densities reached

of more than 4 OD (at 600 nm and 1 cm path length). These parameters allow complete assessment of a host strain's growth within less than 30 hours.

A convenient host strain for use in the assay methods of the invention is an lcb1 / SLC1-1 strain. More conveniently it will include a selection marker, for example the lcb1 gene may be directly replaced by an amino acid biosynthetic gene (such as LEU2, TRP1 or HIS3) or antibiotic resistance such as Geneticin (G418).

Adapting host cells for sustained growth is for example achieved by enhancing expression of the compensatory mutant SLC1-1 allele. We have surprisingly found that can be achieved by cloning the SLC1-1 gene onto a multi-copy plasmid (pYES2-LEU2d- GPD3-SLC1-1 = pNS149) under control of the glyceraldehyde 3-phosphate dehydrogenase promoter. Use of a multi-copy pGPD-SLC1-1 promoter/gene construct yielded a strain with much improved growth characteristics, improved growth rate, final optical density and resistance to freezing. In summary it provided for the first time a host strain which is robust enough for screening purposes.

The GPD3 is an example of a very strong constitutive promoter in S. cerevisiae. Other glycolytic enzymes such as Phosphoglycerate Kinase (PGK), Enolase 1 (ENO), Pyruvate Kinase (PYK) and Fructose-Bisphosphate Aldolase II FBA are convenient sources of other such promoters.

Therefore in a further aspect of the invention we provide an engineered host strain *S. cerevisiae* (lcb1 / pGPD-SLC1-1).

The invention will now be illustrated but not limited by reference to the following Examples and Figures:

Examples

Example 1 Construction of the IPC synthase screening strain (lcb1::kanMX, pNS149 (pGPD3-SLC1-1))

(i) Generation of a *LCB1* deletion strain

As *LCB1* is an essential gene, only one allele of a diploid cell can be deleted without loss of survival. Added phytosphingosine can, however, substitute for an intact *LCB1* gene. Technically, one *LCB1* allele of a diploid *S. cerevisiae* strain (JK9-3daa - Kunz, J. et al, Cell, 1993, 73, 585-596) was disrupted using the kanamycin resistance cassette as described by Wach et al, Yeast, 1996, 12, 259-265.

PCR primers used to create the *LCB1* deletion (lcb1::kanMX)

5' Primer :

GCAATGGCACACATCCCAGAGGTTTTACCCAAATCAATACCGATTCCGGCATTTA TTGCAGCTGAAGCTTCGTACGCTGCAG

3' Primer:

CTATTTTTATTTATTAGATTCTTGGCAACAGGCAAGGATGGACTGCTTGACCCGCA TAGGCCACTAGTGGATCTG

Disruption of *LCB1* and its replacement by kanMX was verified by PCR (using primers 5' of the deleted region directed towards the gene and within kanMX facing towards the promoter). Sporulation of the heterozygous diploid (LCB1/lcb1::KanMX) and tetrad dissection yields 2 kanamycin-sensitive colonies per tetrad when grown on YPD (Sherman et al, Methods in Yeast Genetics, 1986, Cold Spring Harbor Laboratory Press, Cold Spring Harbor N.Y. media) without phytosphingosine, however if the ascus is dissected on media containing 10mM phytosphingosine this results in 4 colonies per tetrad, two of which are resistant to kanamycin (and therefore are lcb1::kanMX).

(ii) Generation of a SLC1-1 allele cloned into a multi-copy plasmid

The dominant *SLC1-1* allele was generated from the wildtype allele by PCR regenerating the sequence as described by Nagiec *et al.* (*op cit*). The mutant *SLC1-1* allele differs from the wildtype allele by a single nucleotide which changes Glutamine 44 in the wild-type protein to Leucine in the suppressor protein. According to the literature (Nagiec *et al*, *op cit*) this mutation should rescue the lcb1::kanMX strain, allowing growth on media without added phytosphingosine.

The *SLC1-1* was amplified from genomic DNA by PCR (creating the point mutation via a mismatch in the 5' primer) and cloned into expression plasmids (eg pYES2-Leu2 (Invitrogen), modified by an inserted Leu2 selection marker = pNS144) using BamHI (5') and SphI (3') as insertion sites (to give pNS145). After transformation (5) into lcb1::kanMX (3), (selection SGal-leucine, no phyto-sphingosine added) microcolonies were established after 12 days of incubation proving and confirming the suppressing function of SLC1-1. However, the viability of these transformants was extremely poor and they were not maintainable in liquid culture. Establishment of frozen stocks from the colonies also failed. A similar phenotype was also observed if the homologous SLC1 promoter was used instead of Gal1 (pNS148).

Primers to generate SLC1-1 by PCR. Restriction sites are shown in bold. The point mutation generating Leu 44 is shown underlined in italics

SLC1-1 5'
CGCGGATCCATGAGTGTGATAGGTAGGTTCTTGTATTACTTGAGGTCCGTGTTGGT
CGTACTGGCGCTTGCAGGCTGTGGCTTTTACGGTGTAATCGCCTCTATCCTGTGCA
CGTTAATCGGTAAGCAACATTTGGCTC*T*GTGG

SLC1-1 3'
ACATGCATGCTTAATGCATCTTTTTTACAGATGAACC

(iii) Generation of a GPD3-driven SLC1-1 allele

We postulated that the poor viability of the lcb1::kanMX pNS145 strain might be due to insufficient expression of *SLC1-1*, so increased expression was attempted. We placed the *SLC1-1* gene under control of the glyceraldehyde-3-phosphate dehydrogenase GPD3 (=TDH3), promoter (Norbeck et al, Yeast, 1997, 16, 1519-1534).

The GPD3 promoter was amplified from *S. cerevisiae* chromosomal DNA by PCR and inserted into a HinDIII site of PNS145 (just 5' of the SLC1-1 start ATG) to create plasmid pNS149 which is a further independent aspect of the invention.

PCR primers generating the GPD3 promoter. Restriction sites are shown in bold

PGPD5'
CCC**AAGCTT**GCCGGCACTAGTTCGAGTTTATCATTATCAATACTCGCC

pGPD 3'
GT**AAGCTT**TATTCGAAACTAAGTTCTTGGTG

Transformation (Ito *et al*, J. Bacteriology, 1983, 153, 163-168) of pNS149 into lcb1::kanMX (see 2. above) yielded readily viable colonies, that also grew very well in liquid culture and were able to recover from freeze-storage.

Example 2 The IPC synthase screen

The utility of the lcb1::kanMX pNS149 strain to identify inhibitors of IPC synthase was evaluated using aureobasidinA as a test compound. The lcb1::kanMX pNS149 strain is a further independent aspect of the invention. As shown in Figure 1, the test compound could be readily identified, as predicted. Inhibition by aureobasidinA was very pronounced in the presence of phytosphingosine but absent if no phytosphingosine was added.

Figure 1a

Inhibition of growth by aureobasidinA in strain lcb1::kanMX, pNS149 with added phytosphingosine.

Figure 1b

Inhibition of growth by aureobasidinA in strain lcb1::kanMX, pNS149 without added phytosphingosine.

Claims:

1. A screening assay for identifying a selective IPC synthase inhibitor which assay comprises contacting a test compound with engineered cells whose capability to synthesize sphingolipids depends on the addition of exogenous phytosphingosine and which are capable of sustained growth via compensatory phospholipids, adding phytosphingosine, and determining IPC synthase inhibition by the test compound by reference to any cell growth inhibition.

2. Engineered cells whose capability to synthesize sphingolipids depends on the addition of exogenous phytosphingosine and which are capable of sustained growth via compensatory phospholipids.

3. Cells as claimed in claim 2 wherein the host strain is an lcb1/SLC1-1 strain.

4. Cells as claimed in claim 3 wherein the SLC-1 gene is under the control of the glyceraldehyde 3-phosphate dehydrogenase (GDP3) gene.

5. Cells as claimed in claim 2 wherein the host strain is lcb1/pGPD-SLC-1.

6. S. cerevisiae (lcb1/pGPD-SLC-1).

7. A selective IPC synthase inhibitor identified using the method of claim 1.

Figure 1a

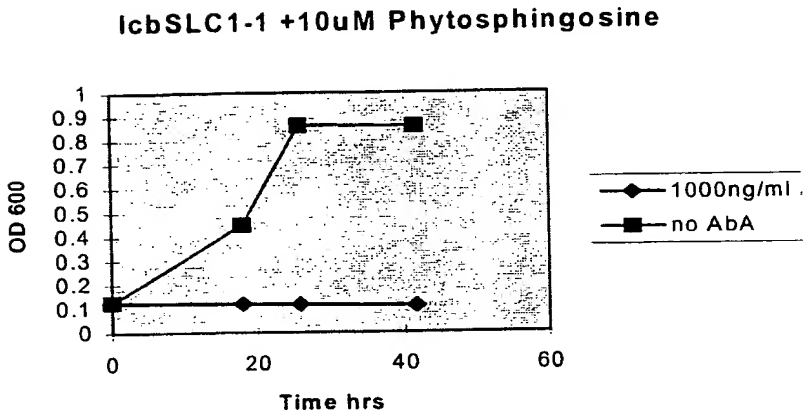
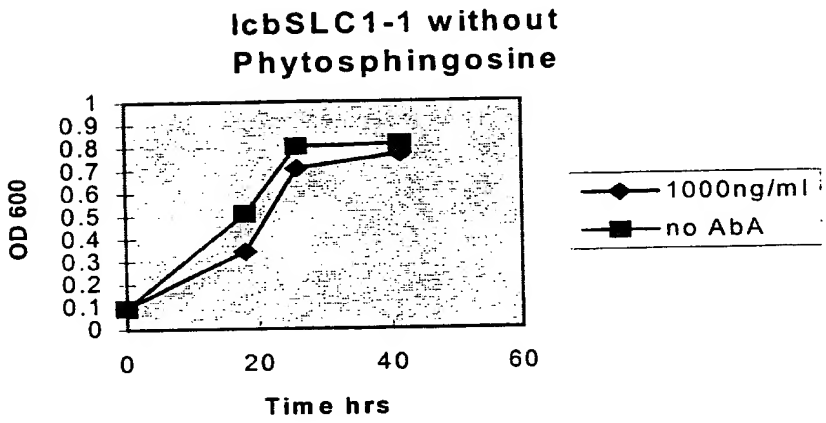

IcbSLC1-1 +10uM Phytosphingosine
OD 600
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
Time hrs
1000ng/ml
no AbA

Figure 1b


IcbSLC1-1 without Phytosphingosine
OD 600
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
Time hrs
1000ng/ml
no AbA

SEQUENCE LISTING

<110> ZENECA Limited

<120> METHOD

<130> NGAP/PHM70429

<140> GB 9825055.8
<141> 1998-11-17

<150> GB 9825055.8
<151> 1998-11-17

<160> 6

<170> PatentIn Ver. 2.1

<210> 1
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<212> DNA
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<220>
<223> Description of Artificial Sequence: 5' PCR primer
for creation of LCB1 deletion

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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 3' PCR primer
for creation of LCB1 deletion

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<212> DNA
<213> Artificial Sequence

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<223> Description of Artificial Sequence: 5' PCR primer
for creation of SLC1-1

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ggtaagcaac atttggctct gtgg 144

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<213> Artificial Sequence

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<223> Description of Artificial Sequence: 3' PCR primer
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<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 5' PCR primer
for creation of GPD3 promoter

<400> 5

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<210> 6
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 3' PCR primer for creation of GPD3 promoter

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INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/GB 99/03789

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/81 C12Q1/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	M M NAGIEC ET AL: "Sphingolipid synthesis as a target for antifungal drugs" JOURNAL OF BIOLOGICAL CHEMISTRY,US,AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 15, no. 272, 1 January 1997 (1997-01-01), pages 9809-9817, XP002077632 ISSN: 0021-9258 cited in the application	1-3,7
A	page 9810, column 1, paragraph 1; figure 9	4-6

☐ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 January 2000	04/02/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gundlach, B

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/GB 99/03789

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/81 C12Q1/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	M M NAGIEC ET AL: "Sphingolipid synthesis as a target for antifungal drugs" JOURNAL OF BIOLOGICAL CHEMISTRY,US,AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 15, no. 272, 1 January 1997 (1997-01-01), pages 9809-9817, XP002077632 ISSN: 0021-9258 cited in the application	1-3,7
A	page 9810, column 1, paragraph 1; figure 9	4-6

☐ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 January 2000	04/02/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gundlach, B

1

Form PCT/ISA/210 (second sheet) (July 1992)

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

PHILLIPS, Neil, Godfrey, Alasdair
AstraZeneca
Global Intellectual Property
P.O. Box 272
Mereside, Alderley Park
Macclesfield, Cheshire SK10 4GR
ROYAUME-UNI

Date of mailing (day/month/year) 18 September 2000 (18.09.00)	
Applicant's or agent's file reference PHM/70429/WO	**IMPORTANT NOTIFICATION**
International application No. PCT/GB99/03789	**International filing date** (day/month/year) 12 November 1999 (12.11.99)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address
ASTRAZENECA UK LIMITED
15 Stanhope Gate
London W1Y 6LN
United Kingdom

State of Nationality: GB
State of Residence: GB
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [ ] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address
ASTRAZENECA AB
S-151 85 Södertälje
Sweden

State of Nationality: SE
State of Residence: SE
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer I. Britel
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

PHILLIPS, Neil, Godfrey, Alasdair
AstraZeneca
Global Intellectual Property
P.O. Box 272
Mereside, Alderley Park
Macclesfield, Cheshire SK10 4GR
ROYAUME-UNI

Date of mailing (day/month/year)
18 September 2000 (18.09.00)

Applicant's or agent's file reference
PHM/70429/WO

IMPORTANT NOTIFICATION

International application No.
PCT/GB99/03789

International filing date (day/month/year)
12 November 1999 (12.11.99)

1. The following indications appeared on record concerning:

[ ] the applicant [ ] the inventor [X] the agent [ ] the common representative

Name and Address

PHILLIPS, Neil, Godfrey, Alasdair
Global Intellectual Property
AstraZeneca UK Limited
Mereside, Alderley Park
Macclesfield
Cheshire SK10 4TG
United Kingdom

State of Nationality:
State of Residence:
Telephone No.: +44-1625-514304
Facsimile No.: +44-1625-583358
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address

PHILLIPS, Neil, Godfrey, Alasdair
AstraZeneca
Global Intellectual Property
P.O. Box 272
Mereside, Alderley Park
Macclesfield, Cheshire SK10 4GR
United Kingdom

State of Nationality:
State of Residence:
Telephone No.: +44-1625-514304
Facsimile No.: +44-1625-583358
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

Authorized officer
I. Britel
Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 27 July 2000 (27.07.00)	
International application No. PCT/GB99/03789	**Applicant's or agent's file reference** PHM/70429/WO
International filing date (day/month/year) 12 November 1999 (12.11.99)	**Priority date** (day/month/year) 17 November 1998 (17.11.98)
Applicant SCHNELL, Norbert, Friedemann et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

16 May 2000 (16.05.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Pascal Piriou
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/331 (July 1992)

GB9903789

PATENT COOPERATION TREATY

16 MAY 2000

Copied for DE

PCT

From the INTERNATIONAL BUREAU

To:

PHILLIPS, Neil, Godfrey, Alasdair
Global Intellectual Property
AstraZeneca UK Limited
Mereside, Alderley Park
Macclesfield
Cheshire SK10 4TG
ROYAUME-UNI

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

Date of mailing (day/month/year) 03 May 2000 (03.05.00)	
Applicant's or agent's file reference PHM/70429/WO	**IMPORTANT NOTIFICATION**
International application No. PCT/GB99/03789	**International filing date** (day/month/year) 12 November 1999 (12.11.99)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address

ZENECA LIMITED
15 Stanhope Gate
London W1Y 6LN
United Kingdom
Macclesfield
Cheshire SK10 4TG
United Kingdom

State of Nationality	State of Residence
GB	GB

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [ ] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address

ASTRAZENECA UK LIMITED
15 Stanhope Gate
London W1Y 6LN
United Kingdom
Macclesfield
Cheshire SK10 4TG
United Kingdom

State of Nationality	State of Residence
GB	GB

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[X] the designated Offices concerned
[ ] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

I. Britel

Telephone No.: (41-22) 338.83.38

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* PHM/70429/WO

Box No. I TITLE OF INVENTION

METHOD

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

ZENECA Limited
15 Stanhope Gate
London
W1Y 6LN

☐ This person is also inventor.

Telephone No. +44-1625-516173

Facsimile No. +44-1625-583358

Teleprinter No. 669095/669388

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

SCHNELL, Norbert Friedemann
Mereside, Alderley Park
Macclesfield, Cheshire
SK10 4TG, GB.

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is,country)* of nationality: DE

State *(that is, country)* of residence: GB

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

PHILLIPS, Neil Godfrey Alasdair
Intellectual Property Department
ZENECA Pharmaceuticals
Mereside, Alderley Park, Macclesfield
Cheshire. SK10 4TG - GB

Telephone No. +44-1625-514304

Facsimile No. +44-1625-583358

Teleprinter No. 669095/669388

☐ **Adress for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

CHAVDA, Jini Suberna
Mereside, Alderley Park
Macclesfield, Cheshire.
SK10 4TG. GB.

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

- [X] **AP** **ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- [X] **EA** **Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- [X] **EP** **European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- [X] **OA** **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- [X] AL Albania .....
- [X] AM Armenia .....
- [X] AT Austria .....
- [X] AU Australia .....
- [X] AZ Azerbaijan
- [X] BA Bosnia and Herzegovina .....
- [X] BB Barbados
- [X] BG Bulgaria .....
- [X] BR Brazil .....
- [X] BY Belarus .....
- [X] CA Canada
- [X] CH and LI Switzerland and Liechtenstein
- [X] CN China .....
- [X] CU Cuba .....
- [X] CZ Czech Republic .....
- [X] DE Germany .....
- [X] DK Denmark .....
- [X] EE Estonia .....
- [X] ES Spain .....
- [X] FI Finland .....
- [X] GB United Kingdom
- [X] GD Grenada
- [X] GE Georgia .....
- [X] GH Ghana .....
- [X] GM Gambia
- [X] HR Croatia .....
- [X] HU Hungary .....
- [X] ID Indonesia
- [X] IL Israel .....
- [X] IN India .....
- [X] IS Iceland
- [X] JP Japan .....
- [X] KE Kenya .....
- [X] KG Kyrgyzstan .....
- [X] KP Democratic People's Republic of Korea .....
- [X] KR Republic of Korea .....
- [X] KZ Kazakhstan .....
- [X] LC Saint Lucia
- [X] LK Sri Lanka
- [X] LR Liberia
- [X] LS Lesotho .....
- [X] LT Lithuania
- [X] LU Luxembourg
- [X] LV Latvia
- [X] MD Republic of Moldova .....
- [X] MG Madagascar .....
- [X] MK The former Yugoslav Republic of Macedonia .....
- [X] MN Mongolia
- [X] MW Malawi .....
- [X] MX Mexico .....
- [X] NO Norway
- [X] NZ New Zealand .....
- [X] PL Poland .....
- [X] PT Portugal .....
- [X] RO Romania
- [X] RU Russian Federation .....
- [X] SD Sudan
- [X] SE Sweden
- [X] SG Singapore
- [X] SI Slovenia .....
- [X] SK Slovakia .....
- [X] SL Sierra Leone
- [X] TJ Tajikistan .....
- [X] TM Turkmenistan .....
- [X] TR Turkey .....
- [X] TT Trinidad and Tobago .....
- [X] UA Ukraine .....
- [X] UG Uganda .....
- [X] US United States of America .....
- [X] UZ Uzbekistan .....
- [X] VN Viet Nam .....
- [X] YU Yugoslavia .....
- [X] ZW Zimbabwe .....

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

- [X] UA Ukraine AE United Arab Emirates MA Morocco .....
- [X] ZA South Africa DM Dominica .....
- [X] CR Costa Rica .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Box No. II PRIORITY CLAIM

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) (17/11/98) 17-Nov-1998	9825055.8	GB		
item (2)				
item (3)				

[X] The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): item (1)

* *Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):*

ISA /

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets**:

request	: 04
description (excluding sequence listing part)	: 06
claims	: 01
abstract	: 01
drawings	: 02
sequence listing part of description	: 03
Total number of sheets	: 17

This international application is **accompanied by** the item(s) marked below:

1. [X] fee calculation sheet
2. [X] separate signed power of attorney
3. [ ] copy of general power of attorney; reference number, if any:
4. [ ] statement explaining lack of signature
5. [ ] priority document(s) identified in Box No. VI as item(s):
6. [ ] translation of international application into (language):
7. [ ] separate indications concerning deposited microorganism or other biological material
8. [X] nucleotide and/or amino acid sequence listing in computer readable form
9. [ ] other *(specify)*:

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: English

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PHILLIPS, Neil Godfrey Alasdair
AGENT FOR APPLICANT

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